

<b>Location:</b> Room W301, Medical Building	<b>Building Number:</b> 181	<b>Date:</b> February 2016	<b>Assessed By:</b> Amber Willems Jones	<b>Health &amp; Safety Representative:</b> Vincé Kalangi
--	--------------------------------	-------------------------------	--	---

**Description of Activity:**

Bacterial Transformation

SWP No. C1V2 Transformation

**Is there past experience with the Activity that may assist in the risk assessment?**

Incidents & Near-hits, Incident Investigations, Workplace Inspections, Training, Standards, Legislation & Codes, Uni Guidance Material, Existing Controls, Industry Standards.

NO

1. TASK	2. RISK	3. Estimated RAW RISK SCORE C x E x L	4. CONTROLS	5. RESIDUAL RISK SCORE				6. RESIDUAL RISK
				C	E	L	CxExL	
Working with antibiotics	May cause an allergic skin reaction	15 x 6 x 1	Personal protective equipment, training	15	6	0.1	9	LOW
Heat Block	Hot surface may cause burns	3 x 4 x 3	Training, correct signage	3	4	0.3	3.6	LOW
Decontamination of waste	Hypochlorite (Diversol) causes skin burns and damage to eyes.	3 x 2 x 1	Prepare disinfectant solutions in fumehood.	3	2	0.1	0.6	LOW
	Ethanol is flammable	1 x 2 x 1	Avoid naked flames	1	2	0.1	0.2	LOW
	<b>TOTAL</b>	<b>110</b>		<b>TOTAL</b>			<b>13.4</b>	<b>LOW RISK</b>
Name and Signature of Laboratory Head/Supervisor or Delegate		Amber Willems Jones				Date		
Name and Signature of Persons Performing Activity or Task						Date		

<b>Number and Title</b>	C1V2 Transformation
<b>Name of Laboratory/ Department</b>	The University of Melbourne IGEM Team Laboratory/ Department of Biochemistry
<b>Author, Date Prepared and Date of Review</b>	<b>Author:</b> Ella Bocquet-Gaylard <b>Updated :</b> February 2016, <b>Date:</b> 22/2/2016 <b>Review by:</b> February 2018
<b>Introduction</b>	Transforming bacterial cells.
<b>Principals/ Scope</b>	This SWP describes the steps to follow in order to transform ligated plasmids into competent E. coli cells.
<b>Risk Management</b>	Risk assessments have been prepared and are available on the Task Based Risk assessment attached to the SWP. <b>Raw Risk:</b> low Residual <b>Risk:</b> low
<b>Safety Management</b>	<b>Hazards:</b> Wear PPE <b>Risk Controls:</b> Low Risk
<b>License/ Permits</b>	N/A
<b>Training/ Competency</b>	All team members must be inducted to the use of any Equipment used.
<b>Equipment</b>	Incubator Shaker Heat Block
<b>Protocol</b>	Materials: Competent Cells LB Broth
<b>Step 1</b>	Thaw the appropriate amount of competent cells on ice. Also pre-chill the required number of empty 1.5mL microcentrifuge tubes.
<b>Step 2</b>	Pipet 50 $\mu$ L aliquots of cells into the pre-chilled tubes.
<b>Step 3</b>	Add 1-5 $\mu$ L of a ligation reaction mixture or 5ng of pure plasmid DNA into each tube. Mix the tube gently.
<b>Step 4</b>	Incubate the tubes on ice for 10 - 30 minutes.
<b>Step 5</b>	Heat shock the tubes for 45 seconds at 42°C. (Similar results are obtained by giving the cells a 2 minute heat shock at 37°C.)
<b>Step 6</b>	Place the tubes immediately on ice for at least 2 minutes.

<b>Step 7</b>	Add 800 $\mu$ L of LB medium to each tube and incubate for 30 min-1 hour at 37°C in the shaking incubator at 200 rpm. Note: when using pure plasmid DNA for the transformation, plate out 100 $\mu$ L of the suspension directly onto LB agar plates containing the appropriate antibiotic.
<b>Step 8</b>	Spin for 1 minute at 6000 rpm.
<b>Step 9</b>	Remove 750 $\mu$ L of the supernatant and resuspend the pellet with the remaining fluid.
<b>Step 10</b>	Plate out the suspension on an LB agar plate containing the appropriate antibiotic.
<b>Controls/ Calibration</b>	N/A
<b>Waste Disposal</b>	Laboratory benches and surfaces used must be decontaminated with 80% ethanol and all bacterial waste must be treated with Hypochlorite before being disposed of in the biohazard bin.
<b>Emergency Procedures</b>	<p><b>First aid measures</b></p> <p>Skin contact: In case of contact, immediately flush skin with plenty of water for at least 20 minutes.</p> <p>Ingestion: Wash out mouth with water. Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Call medical doctor or poison control centre immediately.</p> <p>If any injury occurs notify the laboratory supervisor.</p>
<b>References</b>	
<b>Authorised By</b>	Amber Willems Jones